## REMARKS

The last Office Action of April 1, 2009 has been carefully considered. Reconsideration of the instant application in view of the following remarks is respectfully requested.

Claim 24 is the only claim pending in the application.

Several interviews were had with the Examiner and referred to in prior replies.

Claim 24 currently stands again rejected under 35 U.S.C. § 103 as being unpatentable over Gurunathan in view of Wittig and Makkerh (1916) Current Biology, Vol. 6 (8), 1025-1027.

All formalities have been fulfilled.

## REJECTION OF CLAIM 24 UNDER 35 U.S.C. §103(a) AS BEING UNATENTABLE OVER GURUNATHAN IN VIEW OF WITTIG and MAKKERH

***Examiner's Position***

The Examiner maintained the rejection of claim 24. Gurunathan et al is deemed by the Examiner to be analogous art and is treated as the closest prior art. Gurunathan differs from the invention in that the claimed expression construct is a plasmid, not a MIDGE expression vector, and that the plasmid is not covalently linked to the oligopeptide PKKKRKV. Further, the Examiner asserts that Wittig teaches both differences between Gurunathan and the present invention. The Examiner argues that the disadvantages of plasmids and advantages of MIDGE are also taught by Wittig. Additionally, the Examiner cites Wittig as teaching that any of 3 proposed peptides could be applied to enhance nuclear localisation of DNA expression constructs, including an SV40 NLS peptide, even though Wittig does not disclose or teach the NLS peptide as claimed in claim 24 (SEQ ID NO: 3). However, the Examiner argues, the NLS sequence of SEQ ID NO: 3, was known at the time, as disclosed by Makkerh et al. .

***Applicant's Argument***

Applicant contends that the present invention is non-obvious in light of Gurunathan, Wittig, and Makkerh. The 'modification' of Gurunathan, or rather the implementation of technology which distinguishes the present invention from the prior art, is indeed the use of MIDGE and the addition of the NLS peptide with the specific sequence of PKKKRKV. However, one skilled in the art would not have turned to Wittig to improve the disclosure of Gurunathan. The application of the technology disclosed in Wittig is in fact counterintuitive to how one skilled in the art would proceed from Gurunathan.

Additionally, following the teaching of Wittig produces various outcomes, none of which clearly leads to the claimed invention with expected success.

**a. Gurunathan and Wittig teach opposing lessons**

At the outset, there is no motivation provided by Gurunathan to modify the DNA vaccine as has been carried out in the present invention. In fact, Gurunathan motivates one skilled in the art to improve the DNA vaccine in an **opposite** manner as to what has been carried out in the present invention.

Gurunathan focuses on the induction of IL-12 and IFN-γ production as an essential part of the protective immune response, as mentioned on page 1142, left column,

> *"mice that controlled infection (LACK DNA plus or minus IL-12 DNA or LACK protein plus rIL-12) made substantial amounts of IFN-γ in response to LACK protein, whereas non-healing mice had no detectable IFN-γ."*

And further, also on page 1142, left column,

> *"vaccinated mice treated with anti-IL-12 had a striking inhibition of in vitro production of IFN-γ (Fig. 8 B), suggesting that* ***LACK DNA***

***induced protective immunity through IL-12–dependent production of IFN-γ."*** (Emphasis added)

Any incentive to modify the technology disclosed in Gurunathan would likely involve changes in, or further stimulation of, IL-12 or IFN-γ production. Critically, there is absolutely no mention in Gurunathan of improvement, removal, or replacement of the plasmid vector in order to improve or modify such a DNA vaccine.

Wittig teaches a MIDGE expression construct which essentially strips away all unnecessary sequences from an expression vector, such as bacterial replication origins and coding sequences for other xenogenic proteins (see Wittig, Abstract, last paragraph of column 2 and first two paragraphs of column 3). Regions of plasmid expression vectors that are not essential for the expression of the cloned antigen commonly arise from bacterial sequences. Indeed, the plasmid vector used in Gurunathan as the DNA vaccine (PcDNA-3, Invitrogen, Materials and Methods, page 1138) contains bacterial sequences such as the pBR322 origin of replication, and the Ampicillin and Neomycin resistance genes. Importantly, bacterial DNA sequences are immune stimulatory, and are known to stimulate IL-12 and IFN-γ production. This is also mentioned on page 1140, right column, of Gurunathan,

*"bacterial DNA (by virtue of their immunostimulatory sequences) has been shown to be a potent inducer of IL-12 and IFN-γ".*

Considering IL-12 and IFN-γ stimulation is <u>essential</u> for the protective immune response, one skilled in the art would not consider removing potentially immune stimulating sequences from a DNA vaccine. According to the teaching of Gurunathan, a skilled practitioner may in fact attempt to <u>add</u> extra unnecessary DNA sequence of bacterial origin, in order to increase IL-12 production and thus stimulate the protective immune response. The removal of all unnecessary vector DNA sequences, as carried out in the present invention through the application of MIDGE, is in direct opposition to common knowledge, and in opposition to a likely enhancement of the technology disclosed in Gurunathan.

In the discussion of Gurunathan the importance of immune stimulatory sequences is clearly stated on pages 1143-1144,

> *"Thus, the critical protective dose of subcutaneously administered LACK DNA was due to either a* ***requirement for a threshold dose of immunostimulatory sequence****, antigen encoding sequence, or both".* (Emphasis added)

It is quite unreasonable to assert that it is obvious to modify the technology disclosed in Gurunathan by applying a technology that is contrary to what is suggested within Gurunathan itself. Furthermore, this clearly renders the success of the MIDGE vaccine as unexpected, as the 'modification' of Gurunathan carried out in the present invention went against both suggested enhancement and common knowledge in the field, and still produced an efficient vaccine.

While Wittig does teach some potential disadvantages of excess DNA sequences of bacterial origin in expression vectors, the teaching of Gurunathan portrays such DNA sequences as useful, and even essential, in generating the protective immune response. When trying to develop or improve the prior art as disclosed in Gurunathan, one skilled in the art would not refer to Wittig, as the teaching of Wittig (reduction of bacterial vector sequences) would seem to contradict the teaching of the closest prior art. As argued previously, this combination of prior art (Gurunathan and Wittig) is an artificial product of *ex post facto* analysis, identifiable by the clearly conflicting themes evident in these two very distinct documents.

**b. The application of the PKKKRKV NLS was not obvious from the disclosure in Wittig**

Wittig teaches that peptide chains can be covalently coupled to expression constructs in order to facilitate crossing of the endosomal membrane and nuclear localisation (Wittig et al, column 5). However, Wittig does not teach the specific

peptide sequence used in the present invention (SEQ ID NO: 3) but proposes 3 distinct peptides that could be added to DNA expression constructs; the "nuclear localization sequence from SV40", the "signal peptide from HIV-gp41", and the "23 N-terminal amino acids of haemagglutinine". Use of SEQ ID NO: 3 as a covalently attached NLS peptide in the present invention is not disclosed by Wittig.

If the peptide coupling as taught by Wittig had been applied directly to Gurunathan, various outcomes, and thus uncertainty, would have arisen. It is not predictable which of the 3 proposed peptides would have lead to success. The selection of one of the 3 proposed alternatives cannot be seen as the substitution of one functional equivalent for another, as asserted by the Examiner. Due to the obviously significant difference in the 3 proposed peptides, and the uncertainty in applying any of the three, the selection of the SV40 NLS is certainly not a functional substitution but rather an inventive step.

In considering inventive step, it is irrelevant whether or not the exact NLS peptide sequence was already known (Makkerh). The crucial selective step for the skilled practitioner is to decide from the 3 variants proposed in Wittig, a choice that only leads to uncertainty due to the insufficient disclosure in regards to likely success, and the sequence details of the various peptides.

**c. The declaration of Dr. Timon-Jimenez is sufficient and demonstrates unexpectedness**

In order to demonstrate that the combination of LACK antigen, MIDGE expression vector technology and NLS peptides with the sequence of SEQ ID NO: 3 generated unexpected results, a declaration was made by the inventor Dr. Timon-Jimenez. The Examiner has asserted that the declaration is deficient in two respects. Firstly, that the evidence of unexpected results is not commensurate in scope with the claimed product. Here the Examiner points out that SEQ ID NO: 3 refers to PKKKRKV, whereas the paper referred to by Dr. Timon-Jimenez (Lopez-Fuertes et al. (2202) Vaccine, Vol. 21, 247-257) uses an

NLS sequence of PKKKRKVEDPYC. Secondly, that the results demonstrated are in fact not "unexpected" and that greater, or greater than additive effect is not necessarily sufficient for "unexpectedness".

**d. The functional unit of the NLS sequence is identical in the claimed invention and Lopez-Fuertes et al**

In regards to the first point, the functional unit or the basic peptide of the NLS sequence as applied in the present application is identical to that as referred to by Dr. Timon-Jimenez in the declaration (Lopez-Fuertes et al). As has been demonstrated through genetic approaches, the functional unit of the SV40 NLS is PKKKRKV, which has been shown to be the core requirement for function as a nuclear localisation signal (Kalderon et al, A short amino acid sequence able to specify nuclear location, 1984, Cell 39, 499-509, submitted herewith). Mutations made in and around this defined sequence have demonstrated that the SV40 NLS as stated in SEQ ID NO: 3 is the minimal functional NLS (Kalderon et al., Sequence requirements for nuclear location of simian virus 40 large-T antigen, 1984, Nature 377, 33-38, submitted herewith). Additions to the required NLS sequence can be found in the literature, such as PKKKRKVEDPYC. This sequence has been used in various papers, and such papers are clearly referenced in Lopez-Fuertes (for example reference 27 in Lopez-Fuertes: Zanta et al, Gene delivery: a single nuclear localization signal peptide is sufficient to carry DNA to the cell nucleus, 1999, Proc Natl Acad Sci USA, 96(1):91–6, submitted herewith). This simply constitutes the SV40 NLS with a non-functional additional linker sequence, namely EDPYC.

It is submitted that the wording of the amended claim 24 does not limit the NLS sequence of the present invention to only PKKKRKV, but due to the wording "consisting of" (which was suggested by the Examiner in one interview as means to avoid the references) perhaps it could be argued that the longer NLS sequence (PKKKRKVEDPYC) still falls under claim 24, thus the declaration of unexpectedness would be in commensurate scope with the claimed invention.

Additionally, the use of a linker is common practice when covalently tethering peptides or other signal molecules to any given transport substrate. It could perhaps also be argued that the use of the additional linker-containing NLS sequence in Lopez-Fuertes is simply common practice in the context of 'artificial use' of an NLS (i.e. use outside of the naturally occurring SV40 T-antigen).

**e. The results obtained through the use of MIDGE were unexpected, not due to greater effect, rather due to the application of a distinct expression system with complex features**

In regards to the unexpected nature of the results, the Examiner references multiple passages in Lopez-Fuertes. In doing so, the Examiner points out, and accurately so, that the results obtained through the use if the MIDGE-based vaccine of the present invention (MIDGE-p36-NLS/ MIDGE-p36-NLS) were comparable to the previously known prior art (pMOK-p36/ rVVp36).

As is clearly stated by the Examiner herself, the demonstration of 'greater effect' is not necessarily relevant to 'unexpectedness', because indeed greater effects can be expected. Therefore, we are not able to follow the reasoning of the Examiner in her argument that **'comparable effect'** as shown in the present case demonstrates **'expectedness'**.

The present invention claims a DNA vaccine that has an effectiveness comparable to older known methods, while the DNA vaccine according to the invention is easier to both produce and apply.

However, the issue of expectedness depends not on the comparison of success to older methods, but on the complexity of the attempt, and what would be held to be relevant by one skilled in the art during the development of the present invention. All skilled practitioners in molecular medicine are aware that the replacement of a traditional plasmid with another expression system, namely the application of similar expression machinery in the context of an entirely distinct kind of vector, immediately creates uncertainty as to the likelihood of success.

Generating protective immunity is a complex process, depending on many steps from the administration of DNA expression vectors until the protective immune response. It may have been known that the expression of the p36 antigen from a plasmid vector lead to protective immunisation, but the application of MIDGE and an SV40 NLS peptide demonstrates a clearly novel combination of complex features that surely involves uncertainty when viewed in light of the complexity of the immune system. For these reasons the success of the MIDGE DNA vaccine was indeed unexpected, as declared by Dr. Timon-Jimenez.

Furthermore, the application of MIDGE (essentially a reduction of all DNA sequence unnecessary for antigen expression) would have actually reduced the likelihood of generating a comparable immune response for one skilled in the art (Gurunathan). The removal of non-essential, but immune stimulatory, sequences would be predicted to generate a weaker immune response than full plasmid vectors. Because MIDGE produced a comparable immune response without the non-essential, immune stimulatory sequences, it is likely that the MIDGE vaccine functions via an alternative mechanism or pathway when compared to prior art. For this reason alone, the expectation of success is remote, as would be well understood by a skilled practitioner.

Withdrawal of the rejection of claim 24 as being obvious under 35 U.S.C. §103(a) over Gurunathan, Wittig and Makkerh is thus respectfully requested.

**CONCLUSION**

In conclusion, the combination of Gurunathan and Wittig is only possible when having knowledge of the present invention. Due to the clearly conflicting themes and motivations within Gurunathan and Wittig (as described at length above), one skilled in the art would not have considered modification of the prior art using this combination of teachings. By applying the technology of Wittig, the inventors have taken a counter-intuitive approach, attempting the opposite of

what Gurunathan suggests, and have nevertheless produced an effective vaccine. Due to this reasoning, the present invention must be deemed obvious.

Respectfully submitted,

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